

09/7/3545  
Att #13

=> s amplification or amplified or amplify  
L1 444848 AMPLIFICATION OR AMPLIFIED OR AMPLIFY

=> s ma(w)polymerase  
L2 85655 RNA(W) POLYMERASE

=> s l1 and l2  
L3 2448 L1 AND L2

=> s l3 and py<1990  
1 FILES SEARCHED...  
3 FILES SEARCHED...  
4 FILES SEARCHED...  
L4 219 L3 AND PY<1990

=> dup rem  
ENTER L# LIST OR (END):14  
PROCESSING COMPLETED FOR L4  
L5 119 DUP REM L4 (100 DUPLICATES REMOVED)

=> d l5 ibib abs 1-119

L5 ANSWER 1 OF 119 MEDLINE  
ACCESSION NUMBER: 89333746 MEDLINE  
DOCUMENT NUMBER: 89333746 PubMed ID: 2631699  
TITLE: A PCR artifact: generation of heteroduplexes.  
COMMENT: Comment in: Am J Hum Genet. 1990 Jan;46(1):183-4  
AUTHOR: Nagamine C M; Chan K; Lau Y F  
SOURCE: AMERICAN JOURNAL OF HUMAN GENETICS,  
\*\*\* (1989 Aug)\*\*\* 45  
(2) 337-9.  
Journal code: 3IM; 0370475. ISSN: 0002-9297.  
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L5 ANSWER 2 OF 119 WPIDS COPYRIGHT 2002 DERWENT  
INFORMATION LTD DUPLICATE  
1  
ACCESSION NUMBER: 1989-339975 [46] WPIDS  
CROSS REFERENCE: 1987-306862 [43]; 1988-353543 [49];  
1990-116004 [15];  
1991-369267 [50]; 1997-131797 [12]; 1997-235182 [21]  
DOC. NO. CPI: C1989-150717  
TITLE: Replicable, hybridisable RNA probes - useful e.g. for  
diagnosis of both pathogenic agent and its spectrum of  
antibiotic resistance.  
DERWENT CLASS: B04 D16  
INVENTOR(S): KRAMER, F R; LIZARDI, P M  
PATENT ASSIGNEE(S): (UYCO) UNIV COLUMBIA NEW YORK;  
(UYCO-N) COLUMBIA UNIV  
COUNTRY COUNT: 22  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 8910413	A	19891102 (198946)*	EN	<--	
RW: BE CH DE DK ES FR GB GR IT LI LU NL SE					
W: AU FI JP NO					
PT 90326	A	19891110 (198950)	20	<--	
EP 346594	A	19891220 (198951)	EN	<--	
R: AT BE CH DE ES FR GB GR IT LI LU NL SE					
ZA 8902912	A	19891227 (199006)	<--		
FI 8906113	A	19891219 (199012)	<--		
AU 8935407	A	19891124 (199016)	<--		
NO 8905130	A	19900319 (199017)			
DK 8906351	A	19900205 (199032)			
JP 02504106	W	19901129 (199103)			
NZ 228787	A	19930927 (199341)			
EP 346594	B1	19950531 (199526)	EN	34	
R: AT BE CH DE ES FR GB GR IT LI LU NL SE					

DE 68922882 E 19950706 (199532)  
NO 301776 B1 19971208 (199805)  
CA 1339981 C 19980804 (199842)  
JP 3071796 B2 20000731 (200041) 21

#### APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 346594	A	EP 1989-107037	19890419
ZA 8902912	A	ZA 1989-2912	19890420
JP 02504106	W	JP 1989-505056	19890420
NZ 228787	A	NZ 1989-228787	19890418
EP 346594	B1	EP 1989-107037	19890419
DE 68922882	E	DE 1989-622882	19890419
EP 1989-107037 19890419			
NO 301776	B1	WO 1989-US1673	19890420
NO 1989-5130 19891219			
CA 1339981	C	CA 1989-596864	19890417
JP 3071796	B2	JP 1989-505056	19890420
WO 1989-US1673 19890420			

#### FILING DETAILS:

PATENT NO	KIND	PATENT NO
DE 68922882	E Based on	EP 346594
NO 301776	B1 Previous Publ.	NO 8905130
JP 3071796	B2 Previous Publ.	JP 02504106
Based on WO 8910413		

PRIORITY APPLN. INFO: US 1988-183838 19880420  
AN 1989-339975 [46] WPIDS  
CR 1987-306862 [43]; 1988-353543 [49]; 1990-116004 [15]; 1991-369267 [50];  
1997-131797 [12]; 1997-235182 [21]  
AB WO 8910413 A UPAB: 20000831  
Replicable and hybridisable recombinant single-stranded RNA probe mol.  
(PM) comprises (a) a recognition sequence for the binding of an RNA-directed \*\*\*RNA\*\*\* \*\*\*polymerase\*\*\*, (b) a sequence required for the initiation of prod. strand synthesis by the polymerase, and (c) a heterologous RNA sequence inserted at a specific site in the internal region of the recombinant molecule and complementary to an oligo- or polynucleotide (I) of interest.

USE/ADVANTAGE - The probes may be used to determine the presence or concn. of (I) in a sample and for determining the presence or concn. of several different (I). A specific complex between PM and (I) is formed by hybridisation and after removal of unhybridised PM, incubation with an RNA-directed \*\*\*RNA\*\*\* \*\*\*polymerase\*\*\* effects \*\*\*amplification\*\*\* of the remaining probes. The recombinant-RNA PM's

synthesised are then detected to determine the presence or concn. of (I). In an example, four different RNA's were prepd. by transcription in vitro MDV-1, MDV-Poly, MDV-fal-un and MDV-fal st. Electrophoretic analysis of these transcripts demonstrated that the recombinants which contained probe sequences could be distinguished easily from the other transcripts by their relative mobility. The transcripts were then isolated from the reaction mixt. and used as templates for the synthesis of additional RNA by Qbeta replicase. Kinetic analysis of the amt. of RNA synthesised in the Qbeta replicase reactions demonstrated that both the structured and unstructured recombinant RNA's were excellent templates for

exponential replication. Electrophoretic analysis of the RNA synthesised in each Qbeta replicase reaction, showed that the prods. were homogeneous replicates of the original transcripts.  
0/11

ABEQ EP 346594 B UPAB: 19950705

A replicatable and hybridisable recombinant single-stranded RNA probe molecule comprising: (a) a recognition sequence for the binding of an RNA-directed \*\*\*RNA\*\*\* \*\*\*polymerase\*\*\*; (b) a sequence required

for the initiation of product strand synthesis by the polymerase; and (c) a heterologous RNA sequence complementary to a specific nucleic acid sequence of an infectious agent and inserted at a specific site in the

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1. Document ID: US 6338954 B1

L7: Entry 1 of 24

File: USPT

Jan 15, 2002

DOCUMENT-IDENTIFIER: US 6338954 B1

TITLE: Method for the non-specific amplification of nucleic acid

Abstract Paragraph Left (1):

The present invention is concerned with a method for generating, in a non specific manner, multiple copies of RNA from a pool mRNA's. Such a method is of particular importance in techniques for screening the differences in expression in given cell types or in cells under specific conditions. The present invention provides a non-selective poly A mRNA labeling and amplification method, i.e. a method not encompassing cDNA synthesis. The present invention is directed to a method for amplifying RNA by creating, in a non specific manner, multiple RNA copies starting from nucleic acid containing starting material comprising a pool of mRNA's each mRNA comprising a poly-A tail, wherein the material is contacted simultaneously with an oligonucleotide comprising an oligo-dT sequence, and oligo-dT sequence is blocked at the 3' end, the sequence of a promoter recognized by an RNA polymerase and a transcription initiation region which is located between the oligo-dT sequence and the sequence of the promoter, and further with an enzyme having reverse transcriptase activity, an enzyme having RNase H activity and an enzyme having RNA polymerase activity and the necessary nucleotides and the resulting reaction mixture is maintained under the appropriate conditions for a sufficient amount of time for the enzymatic processes to take place.

Brief Summary Paragraph Right (9):

The present invention provides such a method. The present invention is directed to a method for amplifying RNA by creating, in a non specific manner, multiple RNA copies starting from nucleic acid containing starting material comprising a pool of mRNA's each mRNA comprising a poly-A tail, wherein the material is contacted simultaneously with an oligonucleotide comprising an oligo-dT sequence, the sequence of a promoter recognized by a RNA polymerase and a transcription initiation region which is located between the oligo-dT sequence and the sequence of the promoter, and further with an enzyme having reverse transcriptase activity, an enzyme having RNase H activity and an enzyme having RNA polymerase activity and the necessary nucleotides and the resulting reaction mixture is maintained under the appropriate conditions for a sufficient amount of time for the enzymatic processes to take place.

2. Document ID: US 6291170 B1

L7: Entry 2 of 24

File: USPT

Sep 18, 2001

DOCUMENT-IDENTIFIER: US 6291170 B1

TITLE: Multi-genes expression profile

Brief Summary Paragraph Right (7):

Four steps are used in RAWTS: (1) first strand cDNA synthesis from total RNA or mRNA using oligo(dT) or an mRNA-specific oligo primer, dNTPs, and reverse transcriptase; (2) PCR, wherein one or both primers contain a T7 phage promoter attached to a sequence

complementary to the region to be amplified; (3) transcription of the cDNA strand with T7 RNA polymerase; and (4) reverse transcriptase-mediated dideoxy sequencing of the resultant mRNA transcript.

3. Document ID: US 6271002 B1

L7: Entry 3 of 24

File: USPT

Aug 7, 2001

DOCUMENT-IDENTIFIER: US 6271002 B1

TITLE: RNA amplification method

Brief Summary Paragraph Right (13):

In one embodiment, the invention is directed to a method for amplifying at least one mRNA in a sample containing a plurality of different mRNAs comprising (a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA; (b) synthesizing double-stranded cDNA by contacting under conditions conducive to DNA polymerase activity the first strand cDNA with (i) a first DNA polymerase, and (ii) a second primer that is sufficiently complementary to a sequence 5' to said first primer sequence in said first strand cDNA so as to prime synthesis in a direction toward said first primer sequence; wherein neither said first primer nor said second primer comprises an RNA polymerase promoter sequence in sense or antisense orientation; (c) amplifying the double-stranded cDNA by subjecting the double-stranded cDNA to a single round of polymerase chain reaction (hereinafter "PCR") of 20 cycles or less, wherein DNA is synthesized by use of a second DNA polymerase and a primer pair comprising a forward primer and a reverse primer, said forward primer and said reverse primer each being sufficiently complementary to a different strand of said double-stranded cDNA so as to prime synthesis in a template-dependent manner, wherein said forward primer or reverse primer comprises an RNA polymerase promoter sequence in sense or antisense orientation; and (d) transcribing resultant amplified DNA into cRNA by contacting the amplified DNA with an RNA polymerase specific for said RNA polymerase promoter sequence introduced in step (c) under conditions conducive to RNA polymerase activity, such that cRNA is produced.

Brief Summary Paragraph Right (16):

In another embodiment, the invention is directed to a method for comparing the presence or amount of at least one mRNA of interest in a first sample and in a second sample, said first sample and said second sample each containing a plurality of different mRNAs from one or more cells, comprising: (a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said first sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA; (b) synthesizing double-stranded cDNA by contacting under conditions conducive to DNA polymerase activity the first strand cDNA with (i) a first DNA polymerase, and (ii) a second primer that is sufficiently complementary to a sequence 5' to said first primer sequence in said first strand cDNA so as to prime synthesis in a direction toward said first primer sequence; wherein neither said first primer nor said second primer comprises an RNA polymerase promoter sequence in sense or antisense orientation; (c) amplifying the double-stranded cDNA by subjecting the double-stranded cDNA to a single round of PCR of 20 cycles or less, wherein DNA is synthesized by use of a second DNA polymerase and a primer pair

comprising a forward primer and a reverse primer, said forward primer and said reverse primer each being sufficiently complementary to a different strand of said double-stranded cDNA so as to prime synthesis in a template-dependent manner, wherein said forward primer or reverse primer comprises an RNA polymerase promoter sequence in sense or antisense orientation; (d) transcribing resultant amplified DNA into cRNA by contacting the amplified DNA with an RNA polymerase specific for said RNA polymerase promoter sequence introduced in step (c) under conditions conducive to RNA polymerase activity, such that cRNA is produced; (e) labeling the cRNA produced in step (d) with a first label; (f) repeating steps (a)-(d) with said second sample; (g) labeling the cRNA produced in step (f) with a second label distinguishable from said first label; (h) detecting or measuring the mRNA of interest in the first sample by contacting the cRNA labeled with said first label with a polynucleotide probe capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said probe and said cRNA; (i) detecting or measuring the mRNA of interest in the second sample by contacting the cRNA labeled with said second label with said polynucleotide probe capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said probe and said cRNA; and (j) comparing the mRNA of interest detected or measured in said first sample with the mRNA of interest detected or measured in said second sample.

#### Detailed Description Paragraph Right (61):

In one embodiment, the invention is directed to a method for amplifying at least one mRNA in a sample containing a plurality of different mRNAs comprising (a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in or adjacent to the poly A tail in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA; (b) synthesizing double-stranded cDNA by contacting under conditions conducive to DNA polymerase activity the first strand cDNA with (i) a first DNA polymerase, and (ii) a second primer that is sufficiently complementary to a sequence 5' to said first primer sequence in said first strand cDNA so as to prime synthesis in a direction toward said first primer sequence; wherein neither said first primer nor said second primer comprises an RNA polymerase promoter sequence in sense or antisense orientation; (c) amplifying the double-stranded cDNA by subjecting the double-stranded cDNA to a single round of polymerase chain reaction (hereinafter "PCR") of 20 cycles or less, wherein DNA is synthesized by use of a second DNA polymerase and a primer pair comprising a forward primer and a reverse primer, said forward primer and said reverse primer each being sufficiently complementary to a different strand of said double-stranded cDNA so as to prime synthesis in a template-dependent manner, wherein said forward primer or reverse primer comprises an RNA polymerase promoter sequence in sense or antisense orientation; and (d) transcribing resultant amplified DNA into cRNA by contacting the amplified DNA with an RNA polymerase specific for said RNA polymerase promoter sequence introduced in step (c) under conditions conducive to RNA polymerase activity, such that cRNA is produced.

#### Detailed Description Paragraph Right (80):

In one embodiment, the invention is directed to a method for comparing the presence or amount of at least one mRNA of interest in a first sample and in a second sample, said first sample and said second sample each containing a plurality of different mRNAs from one or more cells, comprising: (a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said first sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in or adjacent to the poly A tail in the mRNA so as to prime synthesis towards the 5' end of the mRNA; (b) synthesizing double-stranded cDNA by contacting under conditions conducive to DNA polymerase activity the first strand cDNA with (i) a DNA polymerase, and (ii) a second primer that is sufficiently complementary to a sequence 5' to said first primer sequence in said first strand cDNA so as to prime synthesis toward said first primer sequence; (c) amplifying the

double-stranded cDNA by subjecting the double-stranded cDNA to a single round of PCR of 20 cycles or less, wherein DNA is synthesized by use of a DNA polymerase and a primer pair comprising a forward primer and a reverse primer, said forward primer and said reverse primer each being sufficiently complementary to a different strand of said double-stranded cDNA so as to prime synthesis in a template-dependent manner, wherein said forward primer or reverse primer comprises an RNA polymerase promoter sequence in sense or antisense orientation; (d) transcribing resultant amplified DNA into cRNA by contacting the amplified DNA with an RNA polymerase specific for said RNA polymerase promoter sequence introduced in step (c) under conditions conducive to RNA polymerase activity, such that cRNA is produced; (e) labeling the cRNA produced in step (d) with a first label; (f) repeating steps (a)-(d) with said second sample; (g) labeling the cRNA produced in step (f) with a second label distinguishable from said first label; (h) detecting or measuring the mRNA of interest in the first sample by contacting the cRNA labeled with said first label with a polynucleotide probe capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said probe and said cRNA; (i) detecting or measuring the mRNA of interest in the second sample by contacting the cRNA labeled with said second label with said polynucleotide probe capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said probe and said cRNA; and (j) comparing the mRNA of interest detected or measured in said first sample with the mRNA of interest detected or measured in said second sample.

4. Document ID: US 6197554 B1

L7: Entry 4 of 24

File: USPT

Mar 6, 2001

DOCUMENT-IDENTIFIER: US 6197554 B1

TITLE: Method for generating full-length cDNA library from single cells

#### Brief Summary Paragraph Right (7):

On the other hand, the generation of amplified antisense RNA (aRNA) has been developed to increase transcriptional copy of specific mRNAs from limited amount of cDNA library. The aRNA can be used for characterization of the expression pattern of certain gene transcripts in cells (O'Dell et al., *BioTechniques* 25: 566-570 (1998)). By incorporating an oligo(dT)<sub>n</sub> primer coupled to a T7 RNA polymerase promoter sequence (oligo(dT)<sub>n</sub>-promoter) during reverse transcription (RT), the single copy mRNA can be amplified up to two thousand folds by aRNA amplification (Eberwine et al., *Proc. Natl. Acad. Sci. USA* 89:3010-3014 (1992)). The aRNAs prepared from single live neuron has been reported to cover 50-75% of total intracellular mRNA population (Eberwine et al., (1992); Crino et al., *Proc. Natl. Acad. Sci. USA* 93: 14152-14157 (1996)), indicating that the prevention of mRNA degradation in cells is required to achieve 100% coverage of mRNA amplification. Although these aRNA synthesis methods lead to the identification of some abundant mRNA markers from single cells, the rare mRNAs may not be assessable by the current aRNA methods (O'Dell et al. (1998)), resulting in low completeness of cDNA library.

5. Document ID: US 6132997 A

L7: Entry 5 of 24

File: USPT

Oct 17, 2000

DOCUMENT-IDENTIFIER: US 6132997 A  
TITLE: Method for linear mRNA amplification

CLAIMS:

26. A kit for use in linearly amplifying mRNA into antisense RNA, said kit comprising: an oligonucleotide promoter-primer comprising an RNA polymerase promoter sequence; and ddNTPs.

6. Document ID: US 6114152 A

L7: Entry 6 of 24

File: USPT

Sep 5, 2000

DOCUMENT-IDENTIFIER: US 6114152 A  
TITLE: Methods for making nucleic acids

Brief Summary Paragraph Right (6):

The invention provides methods and compositions for making nucleic acids. The general methods comprise the steps of adding a known nucleotide sequence to the 3' end of a first RNA having a known sequence at the 5' end to form a second RNA and reverse transcribing the second RNA to form a cDNA. According to one embodiment, the first RNA is an amplified mRNA, the known sequence at the 5' end comprises a poly(T) sequence, the adding step comprises using a polyadenyltransferase to add a poly(A) sequence to the 3' end, and the reverse transcribing step is initiated at a duplex region comprising the poly(T) sequence hybridized to the poly(A) sequence. The resultant cDNA transcript may be single-stranded, isolated from the second RNA and optionally converted to double-stranded cDNA, preferably by a DNA polymerase initiating at a noncovalently joined duplex region. The cDNA may also be transcribed to form one or more third RNAs. In another embodiment, the first RNA is made by amplifying a mRNA by the steps of hybridizing to the poly(A) tail of the mRNA a poly(T) oligonucleotide joined to an RNA polymerase promoter sequence, reverse transcribing the mRNA to form single-stranded cDNA, converting the single-stranded cDNA to a double-stranded cDNA and transcribing the double-stranded cDNA to form the first RNA.

Detailed Description Paragraph Right (10):

In a more particular embodiment, the first RNA is itself made by amplifying an RNA, preferably a mRNA. For example, the first RNA may be made by amplifying a mRNA by the steps of hybridizing to the poly(A) tail of the mRNA a poly(T) oligonucleotide joined to an RNA polymerase promoter sequence, reverse transcribing the mRNA to form single-stranded cDNA, converting the single-stranded cDNA to a double-stranded cDNA and transcribing the double-stranded cDNA to form the first RNA. FIG. 1 is a schematic of this serial mRNA amplification embodiment of the invention, highlighting individual steps of the method:

CLAIMS:

20. A method according to claim 1, wherein the first RNA is made by amplifying a mRNA by the steps of hybridizing to the poly(A) tail of the mRNA a poly(T) oligonucleotide joined to an RNA polymerase promoter sequence, reverse transcribing the mRNA to form single-stranded cDNA, converting the single-stranded cDNA to a double-stranded cDNA and transcribing the double-stranded cDNA to form the first RNA.

7. Document ID: US 6110711 A

L7: Entry 7 of 24

File: USPT

Aug 29, 2000

DOCUMENT-IDENTIFIER: US 6110711 A  
TITLE: Method of defining cell types by probing comprehensive expression libraries with amplified RNA

Detailed Description Paragraph Right (18):

In a more particular embodiment, the first RNA is itself made by amplifying an RNA, preferably a mRNA. For example, the first RNA may be made by amplifying a mRNA by the steps of hybridizing to the poly(A) tail of the mRNA a poly(T) oligonucleotide joined to an RNA polymerase promoter sequence, reverse transcribing the mRNA to form single-stranded cDNA, converting the single-stranded cDNA to a double-stranded cDNA and transcribing the double-stranded cDNA to form the first RNA. FIG. 3 is a schematic of this serial mRNA amplification embodiment of the invention, highlighting individual steps of the method:

CLAIMS:

16. A method according to claim 1, wherein the mRNA is amplified by a method comprising the steps of adding a predetermined nucleotide sequence to the 3' end of a first RNA having a predetermined sequence at the 5' end to form a second RNA and reverse transcribing the second RNA to form a cDNA and wherein the cDNA is single-stranded and converted to a double-stranded cDNA by a method comprising the steps of contacting the RNA with a denaturant and contacting the single-stranded cDNA with a DNA polymerase and an oligonucleotide primer comprising a sequence complementary to the 3' end of the single-stranded cDNA and an RNA polymerase promoter, whereby the DNA polymerase initiates the conversion at a noncovalently joined duplex region of the 3' end of the single-stranded cDNA and the oligonucleotide primer.

17. A method according to claim 1, wherein the mRNA is amplified by a method comprising the steps of adding a known predetermined nucleotide sequence to the 3' end of a first RNA having a predetermined sequence at the 5' end to form a second RNA and reverse transcribing the second RNA to form a cDNA and wherein the cDNA is single-stranded and converted to a double-stranded cDNA by a method comprising the steps of contacting the RNA with a denaturant and contacting the single-stranded cDNA with a DNA polymerase and an oligonucleotide primer comprising a sequence complementary to the 3' end of the single-stranded cDNA and an RNA polymerase promoter comprising SEQ ID NO:1 joined to an upstream flanking sequence of about 3 to 100 nucleotides, whereby the DNA polymerase initiates the conversion at a noncovalently joined duplex region of the 3' end of the single-stranded cDNA and the oligonucleotide primer.

20. A method according to claim 1, wherein the mRNA is amplified by a method comprising the steps of adding a predetermined nucleotide sequence to the 3' end of a first RNA having a predetermined

sequence at the 5' end to form a second RNA and reverse transcribing the second RNA to form a cDNA, wherein the first RNA is made by amplifying a mRNA by the steps of hybridizing to the poly(A) tail of the mRNA a poly(T) oligonucleotide joined to an RNA polymerase promoter sequence, reverse transcribing the mRNA to form single-stranded cDNA, converting the single-stranded cDNA to a double-stranded cDNA and transcribing the double-stranded cDNA to form the first RNA.

8. Document ID: US 6066457 A

L7: Entry 8 of 24

File: USPT

May 23, 2000

DOCUMENT-IDENTIFIER: US 6066457 A  
TITLE: Global amplification of nucleic acids

#### CLAIMS:

25. A method as claimed in claim 24 for global PCR-based amplification of a collection of single stranded cDNA molecules synthesised as first strand cDNA from cellular transcript mRNA, wherein said PCR-amplified cDNA product includes a promoter sequence matched to the selected RNA polymerase, said promoter sequence being incorporated by inclusion in primers used in carving out said first random priming operation and providing at least part of said first known nucleotide sequence.

9. Document ID: US 5922545 A

L7: Entry 9 of 24

File: USPT

13, 1999

DOCUMENT-IDENTIFIER: US 5922545 A  
TITLE: In vitro peptide and antibody display libraries

Detailed Description Paragraph Right (123):  
Polysomes expressing a library of peptides were screened for binding to the D32.39 mAb. An in vitro system was programmed with DNA containing 10.sup.12 different decacodons, incubated and polysomes were isolated and added to microtiter wells containing the immobilized mAb D32.39. Following affinity selection, the bound mRNA was recovered and copied to cDNA using reverse transcriptase and amplified by PCR using primers that included the sequences for the promoter and leader regions of T7 RNA polymerase. A portion of the amplified DNA product was then added to the S30 system for a subsequent round of in vitro synthesis and affinity selection.

10. Document ID: US 5891636 A

L7: Entry 10 of 24

File: USPT

Apr 6, 1999

DOCUMENT-IDENTIFIER: US 5891636 A  
TITLE: Processes for genetic manipulations using promoters

#### Brief Summary Paragraph Right (7):

Four steps are used in RAWTS: (1) first strand cDNA synthesis from total RNA or mRNA using oligo(dT) or an mRNA-specific oligo primer, dNTPs, and reverse transcriptase; (2) PCR, wherein one or both primers contain a T7 phage promoter attached to a sequence complementary to the region to be amplified; (3) transcription of the cDNA strand with T7 RNA polymerase; and (4) reverse transcriptase-mediated dideoxy sequencing of the resultant mRNA transcript.

11. Document ID: US 5869248 A

L7: Entry 11 of 24

File: USPT

Feb 9, 1999

DOCUMENT-IDENTIFIER: US 5869248 A  
TITLE: Targeted cleavage of RNA using ribonuclease P targeting and cleavage sequences

#### Detailed Description Paragraph Right (80):

In order to test the abilities of EGSs derived from the individual variants selected above for CAT mRNA targeting, sequences corresponding to the EGS segment of each chimeric tRNA were amplified by PCR using primers SEC-1A (Sequence ID No. 2) and SEC-1T (TAATACGACTCACTATAGGCCAACTGAGCAGAC, Sequence ID No. 16), which contains a promoter sequence for T7 polymerase, and RNAs were transcribed with T7 RNA polymerase. EGS-directed CAT mRNA cleavage was assayed in 10 .mu.l of 50 mM Tris-Cl, pH 7.5, 10 mM MgCl.sub.2 100 nM NH.sub.4 Cl containing 0.25 pmol (1000 cpm) of substrate RNA and 1 or 5 pmol of EGS RNAs. Reaction mixtures were incubated at 37.degree. C. for 30 min with 10 units of RNase P from HeLa cells, followed by electrophoresis in 5 % polyacrylamide/7M urea gels.

12. Document ID: US 5728521 A

L7: Entry 12 of 24

File: USPT

Mar 17, 1998

DOCUMENT-IDENTIFIER: US 5728521 A  
TITLE: Targeted cleavage of RNA using eukaryotic ribonuclease P and external guide sequence

Detailed Description Paragraph Right (75):  
In order to test the abilities of EGSs derived from the individual variants selected above for CAT mRNA targeting, sequences corresponding to the EGS segment of each chimeric tRNA were amplified by PCR using primers SEC-1A (Sequence ID No. 2) and SEC-1T (TAATACGACTCACTATAGGCCAACTGAGCAGAC, Sequence ID No. 16), which contains a promoter sequence for T7 polymerase, and RNAs were transcribed with T7 RNA polymerase. EGS-directed CAT mRNA cleavage was assayed in 10 .mu.l of 50 mM Tris-Cl, pH 7.5, 10 mM MgCl.sub.2 100 mM NH.sub.4 Cl containing 0.25 pmol (1000 cpm) of substrate RNA and 1 or 5 pmol of EGS RNAs. Reaction mixtures were incubated at 37.degree. C. for 30 min with 10 units of RNAase P from HeLa cells, followed by electrophoresis in 5% polyacrylamide/7M urea gels.

13. Document ID: US 5716785 A

L7: Entry 13 of 24

File: USPT

Feb 10, 1998

DOCUMENT-IDENTIFIER: US 5716785 A  
TITLE: Processes for genetic manipulations using promoters

Brief Summary Paragraph Right (7):  
Four steps are used in RAWTS: (1) first strand cDNA synthesis from total RNA or mRNA using oligo(dT) or an mRNA-specific oligo primer, dNTPs, and reverse transcriptase; (2) PCR, wherein one or both primers contain a T7 phage promoter attached to a sequence complementary to the region to be amplified; (3) transcription of the cDNA strand with T7 RNA polymerase; and (4) reverse transcriptase-mediated dideoxy sequencing of the resultant mRNA transcript.

14. Document ID: US 5670353 A

L7: Entry 14 of 24

File: USPT

Sep 23, 1997

DOCUMENT-IDENTIFIER: US 5670353 A  
TITLE: Subgenomic promoter

Brief Summary Paragraph Right (12):  
It is a further object of the invention to provide a method for the amplified gene expression in transformed cells composed of the steps of introducing into a desired host cell a recombinant DNA molecule containing a subgenomic promoter from a (+) strand RNA virus and a structural gene, wherein expression of the gene is under the regulatory control of the subgenomic promoter and wherein the expression of that gene depends on transcription by host cellular RNA

polymerase II to yield a (-) strand RNA molecule carrying the active form of the subgenomic promoter and subsequent subgenomic transcription of the gene to yield active, (+) sense mRNA by RNA-dependent RNA polymerase activity, and then translation of the subgenomic transcript to produce the polypeptide encoded by the structural gene. The host polII transcription can be controlled by a constitutive, inducible or derepressible polII promoter.

15. Document ID: US 5633447 A

L7: Entry 15 of 24

File: USPT

May 27, 1997

DOCUMENT-IDENTIFIER: US 5633447 A  
TITLE: Plant tissue comprising a subgenomic promoter

Brief Summary Paragraph Right (12):  
It is a further object of the invention to provide a method for the amplified gene expression in transformed cells composed of the steps of introducing into a desired host cell a recombinant DNA molecule containing a subgenomic promoter from a (+) strand RNA virus and a structural gene, wherein expression of the gene is under the regulatory control of the subgenomic promoter and wherein the expression of that gene depends on transcription by host cellular RNA polymerase II to yield a (-) strand RNA molecule carrying the active form of the subgenomic promoter and subsequent subgenomic transcription of the gene to yield active, (+) sense mRNA by RNA-dependent RNA polymerase activity, and then translation of the subgenomic transcript to produce the polypeptide encoded by the structural gene. The host polII transcription can be controlled by a constitutive, inducible or derepressible polII promoter.

16. Document ID: US 5624824 A

L7: Entry 16 of 24

File: USPT

Apr 29, 1997

DOCUMENT-IDENTIFIER: US 5624824 A  
TITLE: Targeted cleavage of RNA using eukaryotic ribonuclease P and external guide sequence

Detailed Description Paragraph Right (71):  
In order to test the abilities of EGSs derived from the individual variants selected above for CAT mRNA targeting, sequences corresponding to the EGS segment of each chimeric tRNA were amplified by PCR using primers SEC-1A (Sequence ID No. 2) and SEC-1T (TAATACGACTCACTATAGGCCAACTGAGCAGAC, Sequence ID No. 16), which contains a promoter sequence for T7 polymerase, and RNAs were transcribed with T7 RNA polymerase. EGS-directed CAT mRNA cleavage was assayed in 10 .mu.l of 50 mM Tris-Cl, pH 7.5, 10 mM MgCl.sub.2 100 mM NH.sub.4 Cl containing 0.25 pmol (1000 cpm) of substrate RNA and 1 or 5 pmol of EGS RNAs. Reaction mixtures were

incubated at 37.degree. C. for 30 min with 10 units of RNAase P from HeLa cells, followed by electrophoresis in 5% polyacrylamide/7M urea gels.

17. Document ID: US 5545522 A

L7: Entry 17 of 24

File: USPT

Aug 13, 1996

DOCUMENT-IDENTIFIER: US 5545522 A

TITLE: Process for amplifying a target polynucleotide sequence using a single primer-promoter complex

Brief Summary Paragraph Right (7):

Four steps are used in RAWTS: (1) first strand cDNA synthesis from total RNA or mRNA using oligo(dT) or an mRNA-specific oligo primer, dNTPs, and reverse transcriptase; (2) PCR, wherein one or both primers contain a T7 phage promoter attached to a sequence complementary to the region to be amplified; (3) transcription of the cDNA strand with T7 RNA polymerase; and (4) reverse transcriptase-mediated dideoxy sequencing of the resultant mRNA transcript.

18. Document ID: US 5466788 A

L7: Entry 18 of 24

File: USPT

Nov 14, 1995

DOCUMENT-IDENTIFIER: US 5466788 A

TITLE: Subgenomic promoter

Brief Summary Paragraph Right (12):

It is a further object of the invention to provide a method for the amplified gene expression in transformed cells composed of the steps of introducing into a desired host cell a recombinant DNA molecule containing a subgenomic promoter from a (+) strand RNA virus and a structural gene, wherein expression of the gene is under the regulatory control of the subgenomic promoter and wherein the expression of that gene depends on transcription by host cellular RNA polymerase II to yield a (-) strand RNA molecule carrying the active form of the subgenomic promoter and subsequent subgenomic transcription of the gene to yield active, (+) sense mRNA by RNA-dependent RNA polymerase activity, and then translation of the subgenomic transcript to produce the polypeptide encoded by the structural gene. The host polII transcription can be controlled by a constitutive, inducible or derepressible polII promoter.

19. Document ID: US 6271002 B1

L7: Entry 19 of 24

File: DWPI

Aug 7, 2001

DERWENT-ACC-NO: 2001-624273

DERWENT-WEEK: 200172

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TITLE: Amplifying and detecting RNA derived from a population of cells by employing a primer that contains an RNA polymerase promoter in a polymerase chain reaction

Basic Abstract Text:

NOVELTY - Amplifying (I) at least one mRNA in a sample containing different mRNAs comprises employing a primer that contains an RNA polymerase promoter in a polymerase chain reaction (PCR).

Basic Abstract Text:

DETAILED DESCRIPTION - Amplifying (I) at least one mRNA in a sample containing different mRNAs comprises employing a primer that contains an RNA polymerase promoter in a polymerase chain reaction (PCR). The method comprises:

Basic Abstract Text (1):

NOVELTY - Amplifying (I) at least one mRNA in a sample containing different mRNAs comprises employing a primer that contains an RNA polymerase promoter in a polymerase chain reaction (PCR).

Basic Abstract Text (2):

DETAILED DESCRIPTION - Amplifying (I) at least one mRNA in a sample containing different mRNAs comprises employing a primer that contains an RNA polymerase promoter in a polymerase chain reaction (PCR). The method comprises:

20. Document ID: AU 200151069 A, WO 200173134 A2

L7: Entry 20 of 24

File: DWPI

Oct 8, 2001

DERWENT-ACC-NO: 2001-616539

DERWENT-WEEK: 200208

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TITLE: Gene expression assay for analyzing gene expression, involves providing array of nucleic acid mixtures at addressable locations on substrate and exposing array to probe for detecting nucleic acid molecules on array

Basic Abstract Text:

(5) producing (M4) a mixture of mRNA-derived nucleic acid molecules, involves isolating an RNA sample from a specimen, obtaining one or more RNA templates from a portion of the RNA sample, hybridizing the templates with a first primer to form a primed template, where the first primer comprises an antisense sequence of an RNA polymerase promoter, synthesizing first strand cDNA from the primed template, hybridizing the first strand cDNA with a second primer to form a switched template, where the second primer has a 5' end and a 3' end and comprises a string of dG residues at the 3' end, synthesizing second strand cDNA from the switched template to generate full-length double stranded cDNA, transcribing antisense (aRNA) from the full-length double stranded cDNA, and reverse transcribing amplified cDNA from the transcribed aRNA;

and

Basic Abstract Text (7):

(5) producing (M4) a mixture of mRNA-derived nucleic acid molecules, involves isolating an RNA sample from a specimen, obtaining one or more RNA templates from a portion of the RNA sample, hybridizing the templates with a first primer to form a primed template, where the first primer comprises an antisense sequence of an RNA polymerase promoter, synthesizing first strand cDNA from the primed template, hybridizing the first strand cDNA with a second primer to form a switched template, where the second primer has a 5' end and a 3' end and comprises a string of dG residues at the 3' end, synthesizing second strand cDNA from the switched template to generate full-length double stranded cDNA, transcribing antisense (aRNA) from the full-length double stranded cDNA, and reverse transcribing amplified cDNA from the transcribed aRNA; and

21. Document ID: US 6197554 B1

L7: Entry 21 of 24

File: DWPI

Mar 6, 2001

DERWENT-ACC-NO: 2001-243448  
DERWENT-WEEK: 200125  
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TITLE: Generating a complete full-length cDNA library from single cells for use in gene chip technology, involves reverse transcribing intracellular mRNAs, adding polynucleotide tail and amplifying formed cDNAs

Basic Abstract Text:

An INDEPENDENT CLAIM is also included for a method (M2) for performing (P) an improved full-length cDNA library synthesis, which involves preventing a number of mRNAs from degradation, where the mRNAs are preserved to be intact in cells, generating a number of cDNAs from the mRNA, where the cDNAs are reverse-transcribed from the mRNAs, permitting the cDNAs to form a number of poly(N)-tailed cDNAs, where the poly(N)-tailed cDNA contains a full-length cDNA sequence flanked with an RNA polymerase promoter in the 5'-end and a polynucleotide tail in the 3'-end and amplifying the poly(N)-tailed cDNAs by a number of promoter- and tail-dependent extension systems, and therefore providing a complete library of full-length cDNAs from the mRNAs.

Basic Abstract Text (10):

An INDEPENDENT CLAIM is also included for a method (M2) for performing (P) an improved full-length cDNA library synthesis, which involves preventing a number of mRNAs from degradation, where the mRNAs are preserved to be intact in cells, generating a number of cDNAs from the mRNA, where the cDNAs are reverse-transcribed from the mRNAs, permitting the cDNAs to form a number of poly(N)-tailed cDNAs, where the poly(N)-tailed cDNA contains a full-length cDNA sequence flanked with an RNA polymerase promoter in the 5'-end and a polynucleotide tail in the 3'-end and amplifying the poly(N)-tailed cDNAs by a number of promoter- and tail-dependent extension systems, and therefore providing a complete library of full-length cDNAs from the mRNAs.

22. Document ID: US 6132997 A

L7: Entry 22 of 24

File: DWPI

Oct 17, 2000

DERWENT-ACC-NO: 2001-006137  
DERWENT-WEEK: 200101  
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TITLE: Improved methods for linearly amplifying mRNA to produce antisense RNA, comprise employing a single reaction mixture where the separation of the double-stranded cDNA from the reverse transcriptase is not required

Basic Abstract Text:

(4) a kit for use in linearly amplifying mRNA into antisense RNA comprising an oligonucleotide promoter-primer having an RNA polymerase promoter sequence, and ddNTPs.

< u>Basic Abstract Text (15):

(4) a kit for use in linearly amplifying mRNA into antisense RNA comprising an oligonucleotide promoter-primer having an RNA polymerase promoter sequence, and ddNTPs.

23. Document ID: US 5716785 A

L7: Entry 23 of 24

File: DWPI

Feb 10, 1998

DERWENT-ACC-NO: 1998-158364  
DERWENT-WEEK: 200174  
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TITLE: Subtractive hybridisation probe production - by generation of cDNA library, nucleic acid amplification, etc.

Basic Abstract Text:

The following are claimed:(1) production of a subtractive hybridisation probe, comprising: (a) synthesising a first double-stranded cDNA collection by treating a first mRNA population with a primer complex, where the primer is complementary to the RNA sequence and is operably linked to a first promoter region for transcription of the cDNA strand complementary to the primer; (b) transcribing the first cDNA into anti-sense RNA by introducing a first RNA polymerase that binds to the first promoter region; (c) hybridising the anti-sense RNA to a second mRNA population, whereby an unhybridised subpopulation of the second RNA population is separated; (d) generating a second double-stranded cDNA collection from the unhybridised subpopulation using a second primer complex comprising a second promoter region in an orientation for transcribing anti-sense RNA complementary to the unhybridised subpopulation, and (e) transcribing the second cDNA into a ribonucleotide probe by introducing a second RNA polymerase that binds to the second promoter region and transcribing RNA which is complementary to the mRNA in the unhybridised subpopulation of the second RNA population; (2) a method for making a cDNA library from a collection of mRNA molecules by: (a) hybridising one or more primer complexes to the mRNAs, where each complex comprises an oligonucleotide primer linked to a promoter sequence; (b) producing a collection of double-stranded cDNAs by extending the primers of any hybridisation duplexes formed between the mRNAs and the complexes, where each double-stranded cDNA



comprises a first cDNA strand which is complementary to one mRNA molecule and operably linked to the promoter, and a second strand which is complementary to the first cDNA strand operably linked to the promoter; (c) transcribing multiple copies of anti-sense RNA from the second strand; and (d) preparing a cDNA library from the anti-sense RNA copies; (3) a process for amplifying at least 1 target nucleic acid sequence, comprising: (a) synthesising a double-stranded nucleic acid by: (i) hybridising a primer complex to the target nucleic acid sequence and extending the primer complex to form a first DNA strand complementary to the target sequence, where the primer complex comprises a promoter and a primer region complementary to the target nucleic acid sequence, and (ii) synthesising a second DNA strand complementary to the first DNA strand without using an exogenous primer complementary to the first DNA strand, and (b) transcribing copies of RNA initiated from the promoter region of the primer complex, where the copies of RNA are complementary to the second DNA strand; (4) a process for detecting expression of at least 1 gene in a preselected cell population, comprising: (a) synthesising double-stranded complementary deoxyribonucleic acid (cDNA) by: (i) hybridising a primer complex comprising a promoter and a polythymidylate (poly(dT)) region complementary to mRNA present in the cell population, (ii) extending the primer complex to form a first cDNA strand, and (iii) synthesising a second cDNA strand complementary to the first cDNA strand without using an exogenous primer complementary to the first cDNA strand; (b) transcribing the double-stranded cDNA into anti-sense RNA (aRNA); and (c) determining the presence of aRNA corresponding to the gene or genes, where the presence of aRNA complementary to a gene corresponds to expression of a gene; (5) a method for subtractive hybridisation comprising: (a) binding a primer to sense RNA molecules in a first population, where the primer is operably linked to a promoter sequence in an anti-sense orientation; (b) synthesising a first complementary DNA (cDNA) strand by elongation from the primer; (c) synthesising a second cDNA strand without using an exogenous primer complementary to the first strand, whereupon a functional promoter is generated; (d) initiating RNA synthesis from the promoter by adding RNA polymerase, whereby anti-sense RNA (aRNA) is produced; (e) introducing the aRNA in molar excess to a second population of sense RNA molecules, whereby complementary RNA sequences from the two populations hybridise, and (f) isolating remaining single-stranded sense RNA; (6) a method for detecting the expression in one or more cells of at least 1 gene, comprising: (a) hybridising a primer complex to RNA molecules in a population of RNA molecules from the cell, where the primer complex comprises a promoter sequence and a primer sequence complementary to at least 1 RNA in the population; (b) synthesising a first complementary DNA (cDNA) strand by elongation from the primer; (c) synthesising a second complementary cDNA strand without using an exogenous primer complementary to the first cDNA strand, where a functional promoter is generated; (d) initiating RNA synthesis from the promoter by adding RNA polymerase, thereby producing amplified anti-sense RNA (aRNA); and (e) detecting the presence of aRNA complementary to the mRNA transcribed from the gene and relating the presence of the aRNA to the expression of the gene in the cell; (7) a method for amplifying a brain cell mRNA, comprising: (a) introducing into a brain cell a reaction mixture comprising: (i) a primer complex operably bound to a promoter sequence in the anti-sense orientation, (ii) reverse transcriptase, and (iii) dATP, dCTP, dGTP, and dTTP, thereby generating a first strand cDNA; (b) harvesting the brain cell; (c) precipitating nucleic acids from the brain cell and recovering the first strand cDNA; (d) synthesising second strand cDNA without using an exogenous primer complementary to the first cDNA strand, and (e) initiating transcription by adding RNA polymerase, rATP, rCTP, rGTP and UTP, whereby anti-sense RNA is produced and a brain cell mRNA is amplified; (8) a process for determining the level of expression of at least 1 mRNA in a preselected cell or cell population relative to the level of expression of the other mRNAs in the same cell or cell population, comprising: (a) adding a primer complex to a mixture comprising a population of mRNAs from the preselected cell or cell population, the primer complex comprising (i) a primer sequence complementary to the mRNAs, and (ii) a promoter sequence in antisense orientation, whereupon the primer complex hybridises to the mRNAs; (b) synthesising double-stranded complementary deoxyribonucleic acid (cDNA) by (i) extending the hybridised primer complexes to produce first strand cDNAs, (ii) synthesising second strand cDNAs without using an

exogenous primer, where the second strand comprises the promoter sequence in sense orientation; (c) transcribing multiple copies of antisense RNA (aRNA) initiated from the promoter region of the primer complexes to produce a population of aRNAs, and (d) analysing the population of aRNAs to determine the level of representation of a specific aRNA sequence relative to other aRNA sequences within the population of aRNAs, where the amount of a specific aRNA in a population of aRNAs corresponds to the amount of the corresponding mRNA in the population of mRNAs, and (9) a process for comparing mRNA expression in different tissues or different physiological states of the same tissue analogically to (8).

#### Basic Abstract Text (1):

The following are claimed: (1) production of a subtractive hybridisation probe, comprising: (a) synthesising a first double-stranded cDNA collection by treating a first mRNA population with a primer complex, where the primer is complementary to the RNA sequence and is operably linked to a first promoter region for transcription of the cDNA strand complementary to the primer; (b) transcribing the first cDNA into anti-sense RNA by introducing a first RNA polymerase that binds to the first promoter region; (c) hybridising the anti-sense RNA to a second mRNA population, whereby an unhybridised subpopulation of the second RNA population is separated; (d) generating a second double-stranded cDNA collection from the unhybridised subpopulation using a second primer complex comprising a second promoter region in an orientation for transcribing anti-sense RNA complementary to the unhybridised subpopulation, and (e) transcribing the second cDNA into a ribonucleotide probe by introducing a second RNA polymerase that binds to the second promoter region and transcribing RNA which is complementary to the mRNA in the unhybridised subpopulation of the second RNA population; (2) a method for making a cDNA library from a collection of mRNA molecules by: (a) hybridising one or more primer complexes to the mRNAs, where each complex comprises an oligonucleotide primer linked to a promoter sequence; (b) producing a collection of double-stranded cDNAs by extending the primers of any hybridisation duplexes formed between the mRNAs and the complexes, where each double-stranded cDNA comprises a first cDNA strand which is complementary to one mRNA molecule and operably linked to the promoter, and a second strand which is complementary to the first cDNA strand operably linked to the promoter; (c) transcribing multiple copies of anti-sense RNA from the second strand; and (d) preparing a cDNA library from the anti-sense RNA copies; (3) a process for amplifying at least 1 target nucleic acid sequence, comprising: (a) synthesising a double-stranded nucleic acid by: (i) hybridising a primer complex to the target nucleic acid sequence and extending the primer complex to form a first DNA strand complementary to the target sequence, where the primer complex comprises a promoter and a primer region complementary to the target nucleic acid sequence, and (ii) synthesising a second DNA strand complementary to the first DNA strand without using an exogenous primer complementary to the first DNA strand, and (b) transcribing copies of RNA initiated from the promoter region of the primer complex, where the copies of RNA are complementary to the second DNA strand; (4) a process for detecting expression of at least 1 gene in a preselected cell population, comprising: (a) synthesising double-stranded complementary deoxyribonucleic acid (cDNA) by: (i) hybridising a primer complex comprising a promoter and a polythymidylate (poly(dT)) region complementary to mRNA present in the cell population, (ii) extending the primer complex to form a first cDNA strand, and (iii) synthesising a second cDNA strand complementary to the first cDNA strand without using an exogenous primer complementary to the first cDNA strand; (b) transcribing the double-stranded cDNA into anti-sense RNA (aRNA); and (c) determining the presence of aRNA corresponding to the gene or genes, where the presence of aRNA complementary to a gene corresponds to expression of a gene; (5) a method for subtractive hybridisation comprising: (a) binding a primer to sense RNA molecules in a first population, where the primer is operably linked to a promoter sequence in an anti-sense orientation; (b) synthesising a first complementary DNA (cDNA) strand by elongation from the primer; (c) synthesising a second cDNA strand without using an exogenous primer complementary to the first strand, whereupon a functional promoter is generated; (d) initiating RNA synthesis from the promoter by adding RNA polymerase, whereby anti-sense RNA (aRNA) is produced; (e) introducing the aRNA in molar

excess to a second population of sense RNA molecules, whereby complementary RNA sequences from the two populations hybridise, and (f) isolating remaining single-stranded sense RNA; (6) a method for detecting the expression in one or more cells of at least 1 gene, comprising: (a) hybridising a primer complex to RNA molecules in a population of RNA molecules from the cell, where the primer complex comprises a promoter sequence and a primer sequence complementary to at least 1 RNA in the population; (b) synthesising a first complementary DNA (cDNA) strand by elongation from the primer; (c) synthesising a second complementary cDNA strand without using an exogenous primer complementary to the first cDNA strand, where a functional promoter is generated; (d) initiating RNA synthesis from the promoter by adding RNA polymerase, thereby producing amplified anti-sense RNA (aRNA); and (e) detecting the presence of aRNA complementary to the mRNA transcribed from the gene and relating the presence of the aRNA to the expression of the gene in the cell; (7) a method for amplifying a brain cell mRNA, comprising: (a) introducing into a brain cell a reaction mixture comprising: (i) a primer complex operably bound to a promoter sequence in the anti-sense orientation, ii) reverse transcriptase, and iii) dATP, dCTP, dGTP, and dTTP, thereby generating a first strand cDNA; (b) harvesting the brain cell; (c) precipitating nucleic acids from the brain cell and recovering the first strand cDNA; (d) synthesising second strand cDNA without using an exogenous primer complementary to the first cDNA strand, and (e) initiating transcription by adding RNA polymerase, rATP, rCTP, rGTP and UTP, whereby anti-sense RNA is produced and a brain cell mRNA is amplified; (8) a process for determining the level of expression of at least 1 mRNA in a preselected cell or cell population relative to the level of expression of the other mRNAs in the same cell or cell population, comprising: (a) adding a primer complex to a mixture comprising a population of mRNAs from the preselected cell or cell population, the primer complex comprising (i) a primer sequence complementary to the mRNAs, and (ii) a promoter sequence in antisense orientation, whereupon the primer complex hybridises to the mRNAs; (b) synthesising double-stranded complementary deoxyribonucleic acid (cDNA) by (i) extending the hybridised primer complexes to produce first strand cDNAs, (ii) synthesising second strand cDNAs without using an exogenous primer, where the second strand comprises the promoter sequence in sense orientation; (c) transcribing multiple copies of antisense RNA (aRNA) initiated from the promoter region of the primer complexes to produce a population of aRNAs, and (d) analysing the population of aRNAs to determine the level of representation of a specific aRNA sequence relative to other aRNA sequences within the population of aRNAs, where the amount of a specific aRNA in a population of aRNAs corresponds to the amount of the corresponding mRNA in the population of mRNAs, and (9) a process for comparing mRNA expression in different tissues or different physiological states of the same tissue analogically to (8).

fragments by electrophoresis

#### Equivalent Abstract Text:

Simultaneous sequence-specific identification of monas comprises (a) isolating a mRNA population, (b) preparing double stranded cDNAs using a mixt. of 12 anchor primers to produce a cDNA sample, (c) cleaving the cDNA sample with the restriction endonuclease MspI and NotI, (d) inserting the cDNA cleaved sample into a vector in such an orientation that is antisense with respect to a T3 promoter within the vector, which is the plasmid pBCSK+ cleaved with ClaI and NotI, (e) transforming E. coli with the vector into which the cleaved cDNA has been inserted to produce cloned inserts, (f) generating linearised fragments of the cloned inserts by different restriction endonuclease treatment, (g) generating a cRNA prepn. of antisense cRNA transcripts by incubation with a T3 RNA polymerase, (h) dividing the prepn. into 16 subpods and transcribing first strand cDNA using a thermostable reverse transcriptase, (i) using the transcription product as a template for PCR, and (j) resolving the PCR amplified fragments by electrophoresis to display bond representing the 3' ends of monas in the sample.

#### Equivalent Abstract Text (1):

Simultaneous sequence-specific identification of monas comprises (a) isolating a mRNA population, (b) preparing double stranded cDNAs using a mixt. of 12 anchor primers to produce a cDNA sample, (c) cleaving the cDNA sample with the restriction endonuclease MspI and NotI, (d) inserting the cDNA cleaved sample into a vector in such an orientation that is antisense with respect to a T3 promoter within the vector, which is the plasmid pBCSK+ cleaved with ClaI and NotI, (e) transforming E. coli with the vector into which the cleaved cDNA has been inserted to produce cloned inserts, (f) generating linearised fragments of the cloned inserts by different restriction endonuclease treatment, (g) generating a cRNA prepn. of antisense cRNA transcripts by incubation with a T3 RNA polymerase, (h) dividing the prepn. into 16 subpods and transcribing first strand cDNA using a thermostable reverse transcriptase, (i) using the transcription product as a template for PCR, and (j) resolving the PCR amplified fragments by electrophoresis to display bond representing the 3' ends of monas in the sample.

24. Document ID: WO 9513369 A1, AU 9510551 A, US 5459037 A, FI 9602000 A, NO 9601902 A, EP 726946 A1, JP 09509306 W, AU 687127

B, AU 9867110 A, US 5807680 A, US 6030784 A, AU 718304 B, US 6096503 A, AU 200047218 A, US 6309834 B1

L7: Entry 24 of 24

File: DWPI

May 18, 1995

DERWENT-ACC-NO: 1995-194088

DERWENT-WEEK: 200173

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TITLE: Simultaneous sequence-specific identification of mRNAs - by transcribing cDNA from the mRNA, carrying out PCR and resolving

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L7: Entry 18 of 24

File: USPT

Nov 14, 1995

US-PAT-NO: 5466788

DOCUMENT-IDENTIFIER: US 5466788 A

TITLE: Subgenomic promoter

DATE-ISSUED: November 14, 1995

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ahlquist; Paul G.	Madison	WI		
French; Roy C.	Lincoln	NE		

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Mycogen Plant Science, Inc.	San Diego	CA			02

APPL-NO: 8/ 296080 [PALM]

DATE FILED: August 25, 1994

## PARENT-CASE:

This is a continuation of application Ser. No. 08/197,222, filed Feb. 16, 1994, now abandoned, which is continuation of application Ser. No. 08/069,457, filed Jun. 1, 1993, now abandoned, which is a continuation of application Ser. No. 07/683,516, filed Apr. 8, 1991, now abandoned, which is a continuation of application Ser. No. 07/168,691, filed Mar. 16, 1988, now abandoned, and a continuation-in-part of Ser. No. 07/368,939, filed Jun. 19, 1989, now abandoned, which is continuation of Ser. No. 06/709,181, filed Mar. 7, 1985, now abandoned.

INT-CL: [6] C12 N 15/11, C12 N 15/82

US-CL-ISSUED: 536/24.1; 536/23.1, 435/172.3

US-CL-CURRENT: 536/24.1; 536/23.1

FIELD-OF-SEARCH: 435/172.1, 435/172.3, 536/23.1, 536/24.1, 935/34, 935/35

## PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

☐ Search Selected☐ Search ALL

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> 4820639	April 1989	Gehrke	435/68

## OTHER PUBLICATIONS

Gallie et al. (1987) Nucleic Acid Research, vol. 15 #21 pp. 8693-8711.  
Vloten-Dating et al. (1985) Plant Molecular Biology 4:pp. 323-326.

**WEST**

L7: Entry 2 of 24

File: USPT

Sep 18, 2001

US-PAT-NO: 6291170

DOCUMENT-IDENTIFIER: US 6291170 B1

TITLE: Multi-genes expression profile

DATE-ISSUED: September 18, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Van Gelder; Russell N.	Des Peres	MO		
Von Zastrow; Mark E.	San Carlos	CA		
Barchas; Jack D.	New York	NY		
Eberwine; James D.	New York	NY		

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE	CODE
Board of Trustees of Leland Stanford University	Stanford	CA				02

APPL-NO: 9/ 286533 [PALM]

DATE FILED: April 5, 1999

## PARENT-CASE:

RELATED APPLICATIONS This is a continuation of application Ser. No. 08/922,965, filed Sep. 3, 1997, now U.S. Pat. No. 5,891,636, which is a continuation of application Ser. No. 08/636,748, filed on Apr. 19, 1996, now U.S. Pat. No. 5,716,785; which is a continuation of application Ser. No. 07/957,647, filed on Oct. 5, 1992, now U.S. Pat. No. 5,545,522; which is a continuation of application No. 07/411,370 filed Sep. 22, 1989, now abandoned.

INT-CL: [7] C12 Q 1/68, C12 N 1/63, C07 H 21/04

US-CL-ISSUED: 435/6; 435/320.1, 536/24.31

US-CL-CURRENT: 435/6; 435/320.1, 536/24.31

FIELD-OF-SEARCH: 536/23.1, 536/24.31, 435/6, 435/320.1

PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<u>Re34069</u>	September 1992	Koster	
<u>3531258</u>	September 1970	Merrifield	